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Research Report

The effects of unilateral eighth nerve block on fictive VOR in the turtle

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ARTICLE INFO

Article history:
Accepted 31 March 2006
Available online 24 May 2006

Keywords:
Nerve recording
Nerve block
Abducen
Trochlear
Oculomotor
In vitro

ABSTRACT

Multiunit activity during horizontal sinusoidal motion was recorded from pairs of oculomotor, trochlear, or abducens nerves of an in vitro turtle brainstem preparation that received inputs from intact semicircular canals. Responses of left oculomotor, right trochlear and right abducens nerves were approximately aligned with leftward head velocity, and that of the respective contralateral nerves were in-phase with rightward velocity. We examined the effect of sectioning or injecting lidocaine (1–2 μL of 0.5%) into the right vestibular nerve. Nerve block caused a striking phase shift in the evoked response of right oculomotor and left trochlear nerves, in which (rightward) control responses were replaced by a smaller-amplitude response to leftward table motion. Such "phase-reversed" responses were poorly defined in abducens nerve recordings. Frequency analysis demonstrated that this activity was advanced in phase relative to post-block responses of the respective contralateral nerves, which were in turn phase-advanced relative to pre-block controls. Phase differences were largest (~10°) at low frequencies (~0.1 Hz) and statistically absent at 1 Hz. The phase-reversed responses were further investigated by eliminating individual canal input from the left labyrinth following right nVIII block, which indicated that the activation of the vertical canal afferents is the source of this activity.

1. Introduction

Compensatory eye movements in response to head rotation are largely generated by the vestibular ocular reflex (VOR). Angular acceleration of the head is transduced by the semicircular canals, and the resulting signal is delivered to the vestibular nuclei in the brainstem via the eighth cranial nerve (nVIII) (Wilson et al., 1976). These brainstem neurons project directly to extraocular motoneurons whose axons form the oculomotor, trochlear, and abducens nerves. The VOR generates counter-rotation of the eyes during head rotation so that gaze is stabilized. Proper functioning of the VOR is critical not only for visual perception but also for equilibrium and balance, demonstrated by the devastating clinical symptoms that result from vestibular insult (Curthoys and Halmagyi, 1995; Dieringer, 1995; Smith and Curthoys, 1989).

Although a three-neuron arc describes the broad organization of the VOR, the reflex exhibits a more complex functional anatomy among the six nuclei that contain motoneurons for the extraocular muscles and depends on the lateral or frontal eye positions of each species (Ezure and Graf, 1984). In general, the trochlear and abducens motoneurons receive contralateral excitation from the vestibular nuclei

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doi:10.1016/j.brainres.2006.03.121

that mediates horizontal VOR. The medial rectus motoneurons, on the other hand, receive excitatory drive from interneurons in the contralateral abducens nucleus. Thus, horizontal responses from trochlear and abducens motoneurons are out-of-phase with that of medial rectus motoneurons.

An active area of investigation is the role of bilateral interactions in tuning the response. The six semicircular canals are organized as three complementary pairs, such that head motion that causes excitation of a given canal also results in inhibition of drive from the contralateral coplanar canal. Input from both canals converges in the vestibular nucleus (Markham et al., 1977; Precht et al., 1973), forming the "push–pull" model of vestibular processing first proposed by Shimazu and Precht (1966). According to this model (see recent reviews Straka and Dieringer, 2004; Uchino et al., 2005), second-order neurons that respond to ipsiversive motion (called Type I cells in the vestibular nucleus) are inhibited by signals that respond to contraversive motion (either Type II cells in the same vestibular nucleus or direct commissural inhibitory projections; Holler and Straka, 2001; Uchino et al., 1986). By combining sensory signals to each vestibular neuron from both sides of the head such that excitatory and inhibitory inputs are out-of-phase with each other, the range and sensitivity is increased and the fidelity of the response is improved.

The "push–pull" model predicts that if nVIII afferents are blocked unilaterally, the response gain would decrease without an effect on response phase. However, our previous findings suggested otherwise (Ariel et al., 2004). There, bilateral processing was investigated by recording unit activity in the vestibular nucleus during horizontal rotation in a novel in vitro turtle brainstem preparation. Because of the turtle's remarkable resistance to hypoxia, the vestibular brainstem and semicircular canals can be studied in isolation and remain viable for several days (Fan et al., 1997). This preparation affords the high degree of experimental control typical of in vitro methodology, yet, because the semicircular canals remain functional, the preparation responds to natural vestibular stimulation. Moreover, during cranial nerve recordings, stable responses can be measured during head pitch, a variety of surgical manipulations and a broad range of frequencies.

Our previous finding was that nVIII block tended to decrease the spike responses of type I units in the vestibular nucleus yet increase the responses of type II units, both ipsilateral to the blocked nerve (Ariel et al., 2004). Furthermore, approximately 50% of type-II cells exhibited a latent ipsiversive response that was revealed by contralateral nVIII block. In order to better understand the bilateral vestibular interactions suggested by these results, we sought to establish whether this activity is apparent in the motor output of the VOR, and, if so, to characterize its frequency response and identify its underlying physiological source.

This study examines the effects of nVIII block on the output of the VOR from the in vitro turtle brainstem. Multiunit activity was recorded in the oculomotor (nIII), trochlear (nIV), and abducens (nVI) nerves using suction electrodes during responses evoked by rotation (Fig. 1). Our results indicate that the response changes previously observed in the vestibular nucleus persist in the output of the reflex arc and reflect vertical canal input, although these effects are differentially distributed among the three motor nuclei.

2. Results

2.1. *In vitro responses of cranial nerves to natural stimulation*

The oculomotor, trochlear, and abducens nerves exhibited a robust response to sinusoidal horizontal table motion, consistent with a bilateral vestibular ocular reflex. As shown in the representative data in Fig. 2, evoked activity was clearly distinguishable from spontaneous background activity. Responses tended to be spindle-shaped and were in phase with table velocity (Tach; lowest trace in Fig. 2). Maximum responses in the left oculomotor, right trochlear and right abducens nerves occurred during leftward table velocity, with the right oculomotor, left trochlear and left abducens nerves demonstrating peak response during motion to the right. Pauses in evoked activity, that would suggest fictive nystagmus, were never apparent in the evoked response, even when the preparation was rotated a complete revolution or more.

Although responses were relatively consistent across stimulus cycles, amplitude variation was observed in different nerves and, to a lesser extent, between left and right nerve recordings in a given pair. These discrepancies are in part predicted by the size of the three nerves, with axon counts of 1500, 600, and 350 typical for the oculomotor, trochlear, and abducens nerves in the turtle (unpublished data). However, this does not account for amplitude variability occasionally observed in contralateral homonymous nerve. These differences were not recording artifacts, as they were not eliminated by impedance-based amplitude corrections (Stys et al., 1991). Rather, they probably reflect variability in the number of axons sampled in a given recording by the suction electrode.

The gross alignment of nerve responses with table velocity rather than position is somewhat unexpected given that this activity drives extraocular muscles to regulate eye position. Such a response may indicate that velocity-to-position integration of the vestibular signal may have been compromised in these in vitro experiments (Cannon and Robinson, 1987). Therefore, nerve responses were evoked by constant velocity rotation, and the time constant of this activity compared to that reported for nVIII afferents in this species.

Typical responses to constant velocity steps are shown in Fig. 3A. These traces depict 45 s of activity recorded from the right (top) and left (bottom) trochlear nerves during application of leftward and rightward velocity steps (duration: 20 s; peak velocity 25°/s). The responses exhibit a rapid rise following motion onset and a slow decay as motion continues. A two-component exponential curve was fit to these data (gray traces in Fig. 3A) and yielded time constants of 0.75 ± 0.24 and 16 ± 2.7 s (n = 8; results lumped from right and left nerves from 4 preparations). The 16-s time constant is considerably greater than the ~3-s time constant measured in vestibular afferents in an in vitro turtle preparation (Brichta and Goldberg, 2000).